\TENT COOPERATION TRI Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 January 2001 (24.01.01)	
International application No. PCT/GB00/02007	**Applicant's or agent's file reference** SMC 70539/WO
International filing date (day/month/year) 25 May 2000 (25.05.00)	**Priority date** (day/month/year) 04 June 1999 (04.06.99)
Applicant BROWN, Richard, John et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 December 2000 (08.12.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Olivia TEFY

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SMC 70539/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/02007	25/05/2000	04/06/1999
Applicant		
AVECIA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02007

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K1/04 C07K14/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 884 327 A (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 16 December 1998 (1998-12-16) example 1 ---	8
X	WO 95 00540 A (R WEBBER) 5 January 1995 (1995-01-05) figure 1 ---	8
A	WO 95 00165 A (SELECTIDE CORPORATION) 5 January 1995 (1995-01-05) the whole document ---	1-14
X	WO 94 02506 A (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 3 February 1994 (1994-02-03) figure 3 ---	8

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	13/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02007

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 360 062 A (BAYER AG) 28 March 1990 (1990-03-28) the whole document -----	1-14

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]nation on patent family members

[redacted]ternational Application No
[redacted]CT/GB 00/02007

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 884327 A	16-12-1998	JP 11124396 A	11-05-1999
WO 9500540 A	05-01-1995	NONE	
WO 9500165 A	05-01-1995	AU 689116 B	26-03-1998
		AU 7114594 A	17-01-1995
		CA 2165780 A	05-01-1995
		EP 0751779 A	08-01-1997
		JP 9500111 T	07-01-1997
		NZ 268292 A	22-09-1997
		US 5635598 A	03-06-1997
WO 9402506 A	03-02-1994	AU 4715493 A	14-02-1994
		DE 69315842 D	29-01-1998
		DE 69315842 T	09-04-1998
		EP 0652896 A	17-05-1995
		ES 2111167 T	01-03-1998
		US 5882645 A	16-03-1999
EP 360062 A	28-03-1990	DE 3831708 A	22-03-1990
		JP 2115226 A	27-04-1990
		US 5049651 A	17-09-1991

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Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/75171 A1

(54) Title: PROCESS FOR THE PREPARATION OF SUPPORTS FOR SOLID PHASE SYNTHESIS

(57) Abstract: A method for preparing a solid support material for carrying out a chemical reaction, said method comprising the following steps: (i) reacting an amino functionalised solid material with a carboxylic acid having at least two similarly protected amino groups to form amide bonds between them, (ii) removing protecting groups in a single step, (iii) optionally repeating steps (i) and (ii) one or more times using the product of the preceding step as the amino functionalised solid material, and (iv) connecting a linkage agent to at least some of the free NH_2 groups of the product. The method increases the loading capacity of the solid support material. It is particularly useful in connection with peptide synthesis.

PROCESS FOR THE PREPARATION OF SUPPORTS FOR SOLID PHASE SYNTHESIS

The present invention relates to a method of preparing support materials useful in solid phase chemical synthesis processes together with the solid materials produced thereby and intermediates used in the process. The support materials are useful in solid phase synthetic processes for the production of a variety of organic molecules, in particular peptides.

The multi-stage synthesis of an organic molecule typically involves numerous isolation steps to separate intermediates, produced at each stage, before progressing to the subsequent stage. These intermediates often require purification to remove excess reagents and reaction by-products and will include procedures such as precipitation, filtration, bi-phase solvent extraction, solid phase extraction, crystallisation and chromatography.

If the reaction chemistry is well defined, many of the isolation procedures used in solution phase synthesis are avoided by reversibly attaching the target molecule to a solid support in a way analogous to the use of a protecting group in a traditional synthesis. Excess reagents and some of the side-products can thereby be removed by filtration and washing of the solid support. Providing that the reactions are efficient and no solid support is lost, the target molecule is recovered in essentially quantitative yield, an objective rarely achieved in solution phase synthesis. In addition the time required to perform operations on a solid support is generally accepted to be a fifth of that required to carry out the equivalent stage in a solution phase synthesis. Another advantage of the solid phase approach is that the whole assembly is carried out in a single reactor.

There are disadvantages to the solid phase approach however. In particular, commercially available supports commonly used for solid phase synthesis of peptides allow a comparatively low loading (<1mmol/g) of reagent, resulting in reactions being carried out at a higher dilution than would normally be achievable in solution. To counteract this, reagents used to carry out the stepwise solid phase assembly are normally used in large excess (3-6 equiv.) and although the excesses are readily removed in the solid phase approach this can add an unnecessary burden on the economics of the process.

Some improvements in terms of increasing the loading of the solid support and solid phase peptide synthesis have been reported. Epton, R. et al; (1985); *Int. J. Biol. Macromol.*, 7, 287-298 describes the use of a bead-form phenolic core polymer as a support matrix. Modified forms of this matrix material, in which the phenolic core is condensed with protected tyrosine are described by Epton, R. et al., in *Peptides 1986, Proceedings of the 19th European Peptide Symposium*, Ed., Theodoropolulos, D.; Publ., Walter de Gruyter, Berlin, 1987, p151-154.

Kates et al., Peptide Science, 47, 5, 1998, 365-380 describes the introduction of polyethylene glycol into solid phase supports to increase the hydrophilicity of the support. Ornithine which is differently protected on each amino group was added to amino functionalised polystyrene, and one protecting group removed so as to allow for bond formation with polyethylene glycol (PEG) by way of a carboxylic acid group which is thereby introduced into the chain. This increases the number of available linking groups, but the loading of the resins was only 0.3-0.5mmol/g. Solid phase dendrimer synthesis involving repeated treatments of a functionalised support with methyl acrylate and 1,3-propanediamine to produce a structure branched as a result of the production of tertiary amine groups has been described by Wells et al., Peptide Science, 47 (1998) 385-396.

The applicants have found a method by which a support can be modified using simple process steps to significantly increase the loading capacity, in particular for peptide synthesis, where it is particularly suitable and economic for large scale manufacture of peptides.

The present invention provides a method for preparing a solid support material for carrying out a chemical reaction, the said method comprising the following steps:

(i) reacting an amino functionalised solid material with a carboxylic acid having at least two similarly protected amino groups to form amide bonds between them,

(ii) removing the protecting groups in a single step,

(iii) optionally repeating steps (i) and (ii) one or more times, using the product of the preceding step as the amino functionalised solid material, and

(iv) connecting a linkage agent to at least some of the free NH_2 groups of the product.

The product of this method is an amino functionalised branched amide-containing organic structure with the amino groups connected to a linkage agent.

This method provides a simple means of increasing the loading capacity of a support material without the need for complex chemical processing. The number of sites available to attach to linkage agents increases as a result of the production of branches. The number of branches available in step (iv) will depend upon the number of NH_2 groups present in the final product, and this is a function of the number of free NH_2 groups within the carboxylic acid structure and the number of times step (iii) is repeated.

The carboxylic acid used in the process is suitably an amino acid containing more than one amino group. Conveniently, this may comprise a naturally occurring amino acid such as lysine or ornithine but synthetic acids may also be used.

Suitable protecting groups for use in the process would be understood in the art, as would the means by which they may be added and subsequently removed. Examples of suitable protecting groups include 9-fluorenylmethoxycarbonyl (Fmoc) and tert-butoxycarbonyl (Boc). Protected forms of amino acids such as Fmoc and Boc protected forms are known in the art or they can be prepared using conventional methods.

The coupling reaction (i) and the reaction of step (iv) above are suitably carried out in an organic solvent such as N,N-dimethylformamide (DMF) or N-methylpyrrolidinone (NMP) in the presence of a coupling reagent. Coupling reagents include those known in the art of peptide synthesis, see for example those coupling reagents disclosed by Wellings, D.A.; Atherton, E.; in *Methods in Enzymology*, Publ., Academic Press, New York (1997) incorporated herein by reference, such as those comprising carbodiimides, especially dialkyl carbodiimides such as N,N'-diisopropylcarbodiimide (DIC), and reagents that form active esters, particularly benzotriazole active esters *in situ*, such as 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or benzotriazole-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP), optionally in the presence of a base such as diisopropylethylamine (DIPEA) or N-methylmorpholine (NMM); or any other suitable activating agent common in the art of peptide synthesis.

The deprotection reaction conditions used in step (ii) will depend largely on the nature of the particular protecting group used and will be readily apparent to chemists. For instance, reaction with a base such as piperidine will result in the removal of protecting groups such as Fmoc groups and the reaction is suitably effected in an organic solvent such as N,N-dimethylformamide (DMF) or N-methylpyrrolidinone (NMP).

As used herein, the term "branched amide-containing organic structure" describes organic moieties which have a plurality of optionally substituted hydrocarbyl chains, each of which may be for example of from 2 to 12 suitably from 2 to 8 carbon atoms in length, and may be optionally interposed by a heteroatom, such as oxygen, nitrogen and sulphur. At least some of the chains are linked together by way of amide bonds, formed during the method of the reaction. Each chain of hydrocarbyl atoms may itself be branched. At least some, and preferably substantially all of the chains of the branched structure will carry a linkage agent or a protected form thereof.

Optional substituents on the hydrocarbyl chains may include any group which does not interfere with the subsequent reactions to which the support material will be subjected. Oxo substituents will be present as a matter of course on the hydrocarbyl chains as a result of the formation of amide bonds in the branched structure. Other possible substituents include substituted amines such as di-alkyl amines, further oxo substitutents, ether groups such as alkyl ethers, thioethers such as alkyl thioethers, alkenyls, alkynyls, nitro, halo in particular fluoro and amides such as alkylamides.

Similarly, heteroatoms such as sulphur, oxygen and nitrogen, may be interposed in the hydrocarbyl chains, in addition to the nitrogen atoms present as a result of the formation of the amide bonds, provided they do not interfere with the subsequent reaction.

As used herein, the expression "hydrocarbyl" refers to any structure comprising carbon and hydrogen atoms. For example, these may comprise or be derived from alkyl,

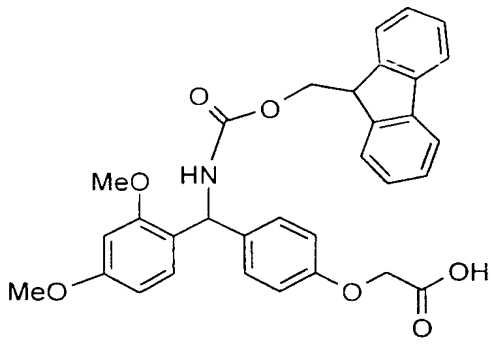
alkenyl, alkynyl, aryl, heterocyclyl, alkoxy, aralkyl, cycloalkyl, cycloalkenyl or cycloalkynyl groups. Groups derived from alkyl groups are for example alkylene groups, and from alkenyl groups are alkenylene groups, etc..

In this specification the term 'alkyl' when used either alone or as a suffix includes structures containing up to 20, preferably up to 10 and more preferably up to 6 carbon atoms. Similarly the terms "alkenyl" and "alkynyl" refer to unsaturated straight or branched structures containing for example from 2 to 20, preferably from 2 to 10 carbon atoms. Cyclic moieties such as cycloalkyl, cycloalkenyl and cycloalkynyl are similar in nature but have at least 3 carbon atoms. Terms such as "alkoxy" comprise alkyl groups as is understood in the art.

The term "halo" includes fluoro, chloro, bromo and iodo. References to aryl groups include aromatic carbocylic groups such as phenyl and naphthyl. The term "heterocyclyl" includes aromatic or non-aromatic rings, for example containing from 4 to 20, suitably from 5 to 8 ring atoms, at least one of which is a heteroatom such as oxygen, sulphur or nitrogen. Examples of such groups include furyl, thienyl, pyrrolyl, pyrrolidinyl, imidazolyl, triazolyl, thiazolyl, tetrazolyl, oxazolyl, isoxazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, quinolinyl, isoquinolinyl, quinoxalinyl, benzothiazolyl, benzoxazolyl, benzothienyl or benzofuryl.

"Heteroaryl" refers to those groups described above which have an aromatic character. The term "aralkyl" refers to aryl substituted alkyl groups such as benzyl.

As used herein, the term "linkage agent" refers to a molecule which can link between the branched solid support material and an assembling molecule to allow it to take part in subsequent reaction procedures, and subsequently be cleaved to release the product undamaged from the support. Such molecules may bear protecting groups such as 9-fluorenylmethoxycarbonyl (Fmoc) and tert-butoxycarbonyl (Boc) which may be removed to allow the linkage agent to take part in a subsequent reaction. Examples of suitable linkage agents are those listed for example in Methods in Enzymology, Publ, Academic Press, New York, Section I, p126-174 (incorporated herein by reference). In particular they will comprise linkage agents used in peptide synthesis and which can couple to amino functionalised supports such as 4-[[1-(9-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (the 'Rink' linkage agent, the group called herein "Fmoc-Linker-Am-OH", of structure (i)),



(i)

as well as 4-hydroxymethylbenzoic acid, 4-hydroxymethylphenoxy acid or any other linkage agents recognised in the art of solid phase synthesis.

The number of branches and therefore the potential number of linkage agents which may be conveniently and advantageously attached to the polymeric material will depend on steric factors, such as the length of the chains within the branched amide-containing structure, as well as to the nature of the linkage agent used and the purpose to which the material is to be put. However, in general, the process is effected such that the support material obtained has from 2-10 branches, such as from 2-8 branches, and preferably about 4-6 branches in each structure. These can be coupled to linkage agents or protected forms thereof, and so be available for synthesis reactions.

One embodiment of the method of the invention, where in step (iii), steps (i) and (ii) are repeated once to yield four free amino groups, is represented schematically in Scheme 1 hereinafter. In this scheme, following representations apply:

S is a solid polymer core, with a single reactive site illustrated;

R^1 is an organic moiety with n + 1 available points for bonding:

R^2 is an organic moiety with m + 1 available points for bonding;

R^3 is either a bond or an organic bridging group;

R^4 and R^5 are protecting groups;

L is a linkage agent, or a protected form thereof;

p is an integer of 1 or more, for example from 1 to 6, provided that p is 1 when R^3 is a bond; and

n and m are independently selected from integers of 2 or more, for example from 2 to 8 and preferably from 2 to 4 and most preferably 2 or 3.

Suitable compounds of formula (I) are well known in the art. The solid polymer core 'S' in formula (I) may comprise polymers such as polyacrylamides, polystyrene or co-polymeric materials as well as inorganic solids such a glass or silica. Some of these reagents have amino functional groups available on the surface. In this case, in compounds of formula (I), R^3 may be a bond, although a spacer group may be added if required. In other cases, the solid material as supplied may comprise different functional groups at the surface and in those cases, amino substitutents must be introduced in a

preliminary step. Methods by which this can be achieved are well known in the art. For example, polymeric materials with acid or ester functionalities at the surface may be reacted with primary or secondary amines as appropriate to form an amide bond and therefore complete the bridging group R^3. In particular, reaction with an amine of formula (X)

$$H_2N\text{-}[(R^8)_zNH)]_y(R^{8'})_x\text{-}NH_2$$
(X)

where x is an integer of 2 or more, suitably from 2 to 6, y is 0 or an integer of 1 or more, suitably from 1 to 5, and each group z is independently 2 or more, for example from 2 to 6, and each R^8 and $R^{8'}$ are the same or different and are optionally substituted divalent hydrocarbyl groups, in particular optionally substituted alkylene groups and especially ($-CH_2-$). In these compounds, y + 1 will be equal to p.

A particular example of a compound of formula (X) are diamines such as those of general formula (XI)

$$NH_2\text{-}(CH_2)_x\text{-}NH_2$$
(XI)

where x is as defined above. A particular example of such an amine is ethylene-diamine.

Where p is greater than 1, the group R^3 will bear more than one amino group and so the number of branches formed during the subsequent reaction stages will increase. Suitably therefore, in the compound of formula (X), y is 1 or more. An example of a multiple amine of formula (X) is diethylenetriamine (i.e. $H_2N\text{-}(CH_2)_2\text{-}NH\text{-}(CH_2)_2\text{-}NH_2$).

Suitable bridging groups R^3 include optionally substituted hydrocarbyl chains of at least two carbon atoms and for example from 2-12 carbon atoms which may itself be interposed with a heteroatom such as oxygen, nitrogen and sulphur. Examples of such groups include, for example, groups of formula $-(CR^9R^{10})_a-$, $-R^{11}\text{-}(CR^9R^{10})_aR^{11}(CR^9R^{10})_b-$, where each R^9 and R^{10} is independently selected from hydrogen or an optional substitutent as described above, and R^{11} is a cycloalkylene or arylene group, and a and b are independently selected from 2 to 12.

Thus the nature of bridging groups R^3 in the compounds of formula (I) to (IX) will depend to some extent upon the particular core polymer S used and the groups required to link to functionalities on the surface. In general, the bridging group will comprise a hydrocarbyl chain linked to the support S and one or more functional group derivatives such as amino, mono- or di-alkylamino or hydroxy groups. Thus an example of a bridging group R^3 is a group of sub formula (XII)

$$-R^{12}(NH)_p-$$
(XII)

where R^{12} is C_{2-6} alkylene optionally interposed with for example oxygen, phenylene; C_{3-8}cycloalkylene or $-(CH_2)_rR^{13}-$ where R^{13} is phenylene or C_{3-8}cycloalkylene, and where p is as defined above.

Particular examples of bridging groups R^3 are

$-(CH_2)_2-NH-$

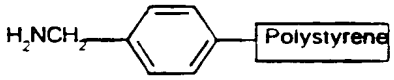
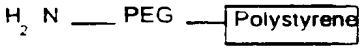
or

$-(CH_2)_2-N(-)-(CH_2)_2NH-$

A particularly preferred compound of formula (I) is derived from a commercially available (Polymer Laboratories) solid, sold under the catalogue name PL-DMA. PL-DMA is prepared by copolymerisation of acryloyl-sarcosine methyl ester, N,N-dimethylacrylamide and bis-acryloylethylenediamine. The procedure for preparation of the polymer core has been described by Atherton, E.; Sheppard, R. C.; In *Solid Phase Synthesis: A Practical Approach*, Publ., IRL Press at Oxford University Press (1984) (incorporated herein by reference). In the commercially available form, the functional loading of the starting polyacrylamide supplied is 1mmol/g. The functional group on the PL-DMA is a methyl ester and this is initially converted to a primary amine functionality by reaction with an alkyl amine as described above.

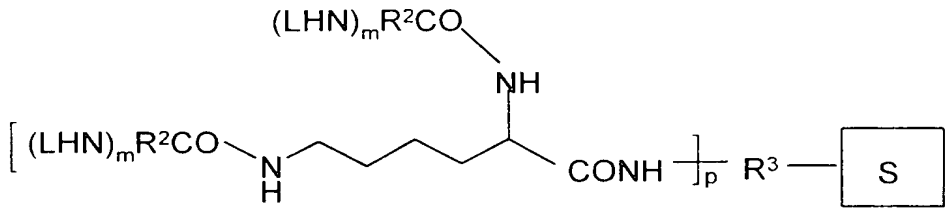
Thus suitable solid supports of formula (I) include those listed in Table 1.

Table 1

Name	Structure	Comments
Aminomethylpolystyrene		Available from most catalogue suppliers (e.g's. Bachem, Novabiochem, Advanced Chemtech).
Aminopolyethyleneglycol-polystyrene		Amino functionalised polyethyleneglycol (PEG) on a polystyrene core is commercially available under the trade name Tentagel. A similar support is available from Perkin-Elmer.
Amino PEG (PEGA) resin	PEGA resins are a copolymer of dimethyl acrylamide, mono-2-acrylamidoprop-1-yl[2-aminoprop-1-yl]polyethylene glycol and bis-2-acrylamidoprop-1-yl polyethylene glycol.	Commercially available from Novabiochem and Polymer Laboratories.
Pepsyn/Polyhipe resins	N,N-dimethylacrylamide (DMA) supported within a rigid macroporous structure.	Commercially available from Novabiochem
Inorganic supports	Amino functionalised	e.g's controlled pore glass, silica.

Suitable organic moieties for R^1 and R^2 in compounds of formulae (II) and (V) respectively include optionally substituted hydrocarbyl groups which may be optionally interposed with heteroatoms such as oxygen, sulphur and nitrogen, or with carbocyclic or heterocyclic rings. The precise nature of these groups is not critical provided that they are inert in the subsequent application to which the support material can will be placed. Preferably however, the compounds of formula (II) and (V) are protected forms of lysine or ornithine (where n and m are 2), and are preferably protected lysine. Suitable protecting groups R^4 and R^5 are described above and include Fmoc and Boc, but preferably Fmoc.

Thus where the compound of formula (II) is lysine, the completion of Scheme 1 will result in the production of a compound of formula (IXA)

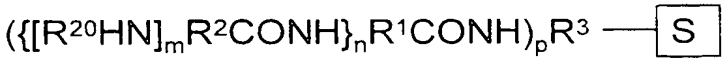


(IXA)

where S, R^3, R^2, L, m and p are as defined above. Where the compound of formula (V) is also protected lysine, m is 2. It can be seen therefore, that where amino acids having two amino groups such as lysine or ornithine are used in the process of the invention, the total number of linkage agents L attached to each available site on the support molecule has been quadrupled following only one operation of step (iii) above. The level of branching can therefore be varied depending upon the number and nature of the amino acid residues interposed between the solid support and the linking groups.

Solid support materials obtainable by the process of the invention are novel and these form a further aspect of the invention. In particular there is provided a compound of formula (IX) as illustrated in Scheme 1, wherein R^1, R^2, R^3, L, n, m and p are as defined above.

The precursors of these compounds (i.e. compounds VI and VII of Scheme 1 wherein R^1, R^2, R^3, n, m and p are as defined above) are also novel and form further aspects of the invention. Thus the invention further provides a compound of formula (XIII)



XIII

wherein S, R^1, R^2, R^3, n, m and p are as defined in above, and R^{20} is hydrogen or an amino protecting group.

In these compounds, preferred values for the variables R^1, R^2, R^3, n., m and p, are as described above.

The solid support materials are suitable for use in the solid phase synthesis of a range of compounds including peptides. Thus in a further aspect, the invention provides a method for preparing a compound, which method comprises binding a reagent to a linkage agent of the support material of the invention, effecting one or more reaction steps to generate product, and thereafter cleaving said product from the support material.

Using a solid support material in accordance with the invention, any required peptide can be prepared, for example using chemistry and techniques employed in traditional Fmoc-based solid phase peptide synthesis (Wellings, D.A.; Atherton, E.; In *Methods in Enzymology*, Publ., Academic Press, New York (1997) (incorporated herein by reference). The peptide can then be cleaved from the support and worked up using standard conditions (Wellings D.A. supra-incorporated herein by reference). Examples of peptides which can be prepared in this way include the peptides described in WO 97/31023 and other therapeutic peptides.

In general peptides will be produced in a stepwise manner as illustrated hereinafter. Specifically, peptides are prepared by coupling a protected amino acid to a linkage agent immobilised on a solid support, deprotecting the amino acid and thereafter coupling a further protected amino acid to said first amino acid and repeating said process until the desired peptide is produced. At the end of the sequence, the peptide is cleaved from the solid support. Suitably the coupling reaction is effected in a solvent such as N,N'-dimethylformamide (DMF) with an activated species prepared using a coupling reagent such as N,N'-diisopropylcarbodiimide in the presence of a compound that forms an active ester with such a coupling agent such as1-hydroxybenzotriazole (HOBt). Cleavage is suitably effected by addition of piperidine, although this will depend to some extent on the nature of the linkage agent as would be understood in the art.

The applicants have found that where the amino acid is a protected 4-aminophenyl acetic acid (PAPA), a preferred coupling agent is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU). Furthermore, a preferred base is diisopropylethylamine (DIPEA). Such reactions, in particular when effected using the support materials described above, form a further aspect of the invention. In this further aspect of the present invention, it is preferred that in couplings where the amino acid is other than a protected 4-aminophenyl acetic acid (PAPA), a preferred method is to use an activated species prepared using a coupling reagent such as a carbodiimide, often a dialkyl carbodiimide, and preferably N,N'-diisopropylcarbodiimide, in the presence of a compound that forms an active ester, such as 1-hydroxybenzotriazole (HOBt).

The invention will now be particularly described by way of example, with reference to the accompanying diagrammatic schemes in which

Scheme 1 is a generalised scheme illustrating the method of the invention, and

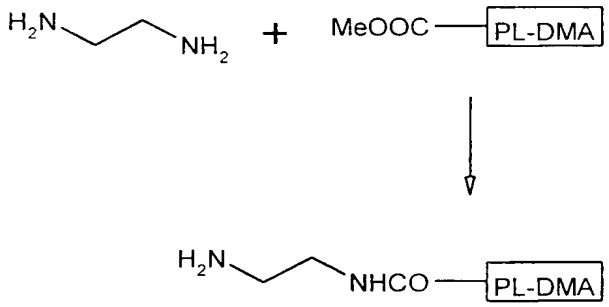
Scheme 2 is a reaction scheme illustrating the preparation of a material of the invention and its use in the production of a target peptide.

Example 1

Preparation of Solid Reaction Support

Step 1

Reaction of ethylene-diamine with PL-DMA

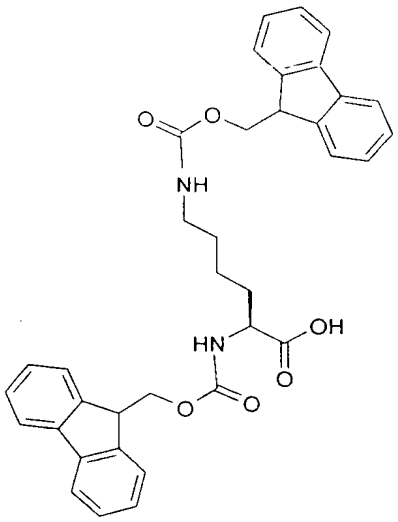


PL-DMA resin (5.00g, 5.00mmol) available from Polymer Laboratories with a molecular weight of 1000, was charged to a 500ml flask equipped with overhead stirrer. Ethylene-diamine (150ml) was added and stirred for 18 hours. The resin derivative was filtered through 500ml sintered glass funnel fitted with a 1L Buchner flask and equipped with overhead stirrer. The filter cake was slurry washed with portions of DMF (60ml) until the pH of the filtrate was <8 as indicated by moistened pH paper.

Step 2

Fmoc-Lys(Fmoc)-OH coupling

N-Hydroxybenzotriazole (HOBt) (2.27g, 16.7mmol, 3.33 equiv.) was charged to a clean dry 250ml flask equipped with a magnetic stirrer. A protected lysine derivative Fmoc-Lys(Fmoc)-OH (7.38g, 12.5mmol, 2.50 equiv.), was added. This compound, of formula,



is commercially available from suppliers of reagents and materials for peptide synthesis.

The contents were then dissolved in DMF (50ml) and the solution chilled to between 0-5°C. Diisopropylcarbodiimide (DIC) (1.75g, 2.18ml, 13.8mmol, 2.75 equiv.) was added whilst keeping the solution below 5°C. The mixture was stirred for 10 minutes at 0-5°C.

A slight nitrogen/argon pressure was applied to the resin product of Step 1, and the mixture added to it with a DMF (5ml) rinse and stir. After 5 minutes, extra DMF (50ml) was added to allow mobilisation of the resin. The mixture was then left for 1 hour. Completion of the reaction was checked by removing approximately 5 mg of beads from the resin bed and carrying out a Kaiser test. In this test, ninhydrin (5% w/v in n-butanol, 6 drops) and phenol (80% w/v in n-butanol, 6 drops) are added to the test tube containing the beads to be tested and heated to 100°C for 5 minutes. A positive result was indicated by a blue colouration of the beads and/or the solution is observed. A negative result was indicated when the blue colour of the beads and solution is not seen. The product was filtered under vacuum and slurry washed with DMF (10 x 50ml).

The Fmoc protecting groups were then removed by addition of piperidine (20% v/v in DMF, 80ml) followed by a vacuum filtration step, a procedure which was effected twice (3 and 7 minute treatments). The product was then washed with DMF (5 x 80ml). After each wash, a vacuum filtration step was effected. HOBt (10% w/v in DMF, 80ml) was added with stirring. After 5 minutes, further DMF was added as required to mobilise the resin which was stirred for a further 15 minutes and then filtered under vacuum.

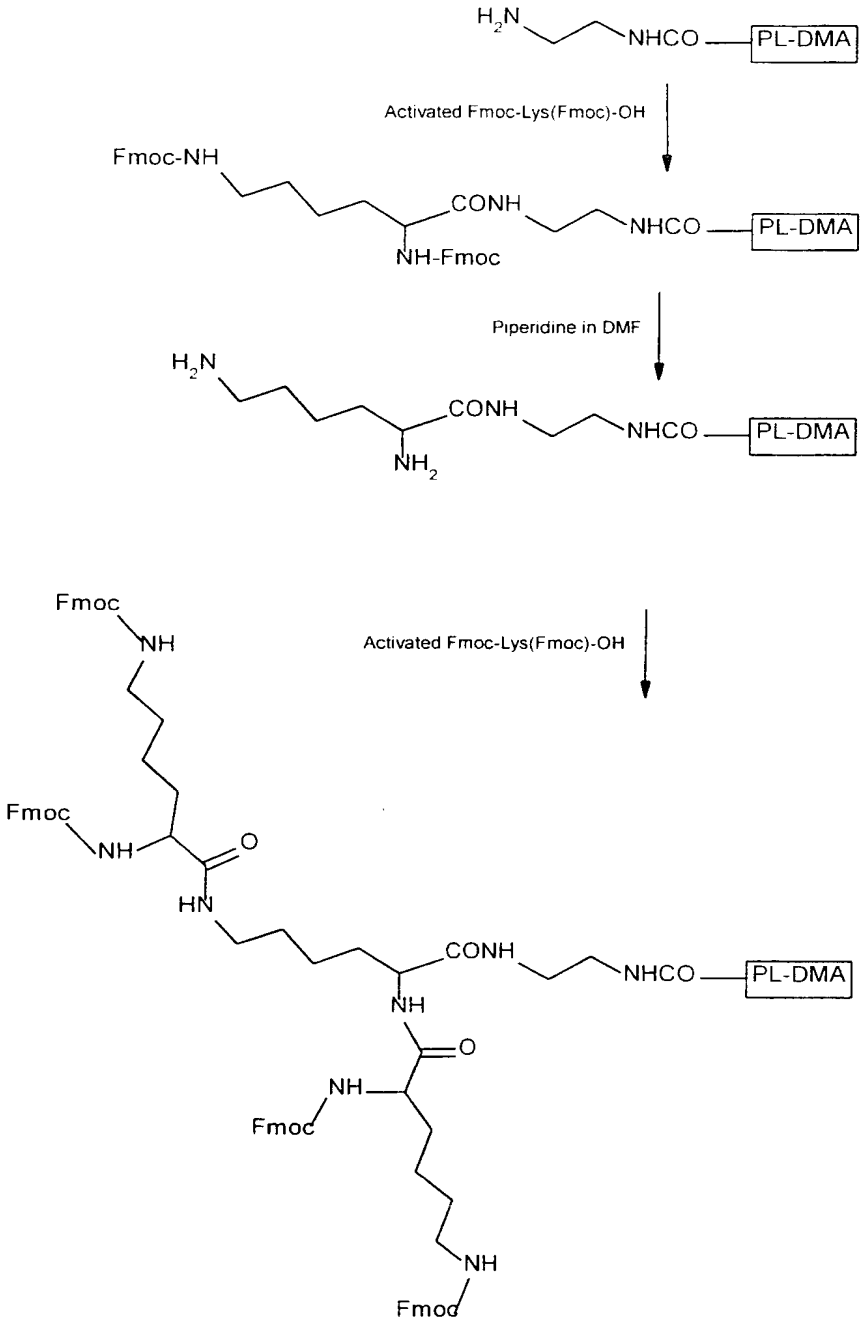
A solution of HOBt (10% w/v in DMF, 80ml) was stirred for 5 minutes after which sufficient extra DMF was added so as to mobilise the resin, which was then stirred for a further 15 minutes. The product was filtered under vacuum and slurry washed with DMF (5 x 80ml) until the filtrate had a pH <8 as indicated by moistened pH paper.

Step 3

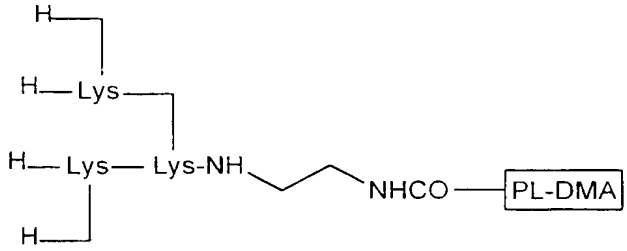
Further Fmoc-Lys(Fmoc)-OH Coupling

To a clean dry 250ml flask equipped with a magnetic stirrer was added HOBt (4.55g, 33.3mmol, 6.67 equiv.) and Fmoc-Lys(Fmoc)-OH (14.8g, 25mmol, 5.0 equiv.) which were dissolved in DMF (75ml). The solution was cooled to 0-5°C and DIC (3.51g, 4.35ml, 27.5mmol, 5.5 equiv.) added whilst keeping the solution below 5°C and stirring for 10 minutes.

A slight nitrogen/argon pressure was applied to the resin from Step 3 and the resultant cooled solution added to the resin with a DMF (5ml) rinse and stirring. After 5 minutes, extra DMF (50ml) was added and the mixture left for 1 hour. Completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test. The product was filtered under vacuum and slurry washed with DMF (10 x 80ml), deprotected using piperidine (20% v/v in DMF) and HOBt added as described in Step 2. The reaction here can be illustrated by the following scheme:



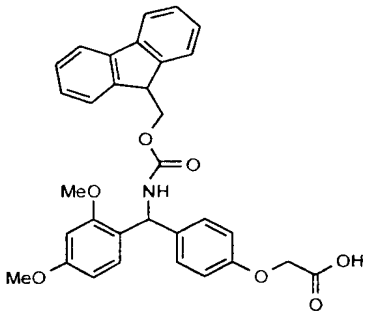
The final deprotected branched lysine construct can be written in the simplified form as follows.



Step 4

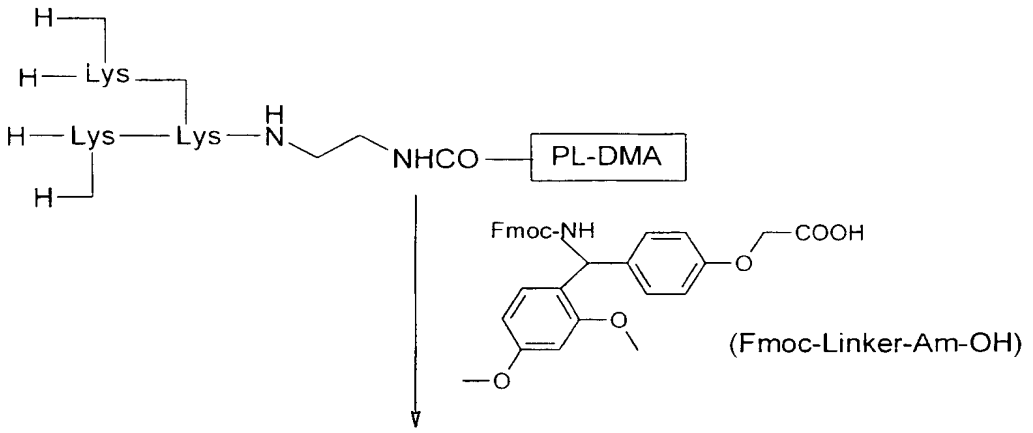
Fmoc-LinkerAm-OH Coupling

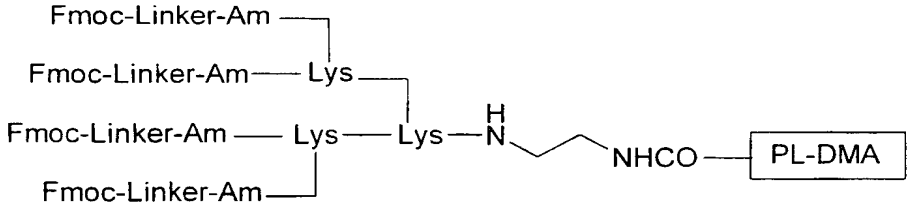
HOBt (9.10g, 66.7mmol, 13.3 equiv.) and Fmoc-LinkerAm-OH (27.0g, 50.0mmol, 10.0 equiv.) were added to a clean dry 250ml flask equipped with a magnetic stirrer and dissolved in DMF (60ml). The Fmoc-LinkerAm-OH was of structure



The solution was chilled to 0-5°C and DIC (7.01g, 8.70ml, 55.0mmol, 11 equiv.) added whilst keeping the solution below 5°C. The mixture was stirred for 10 minutes at 0-5°C, after which it was added to the resin of Step 3 to which a slight nitrogen/argon pressure was added.

This step is illustrated by the following scheme:





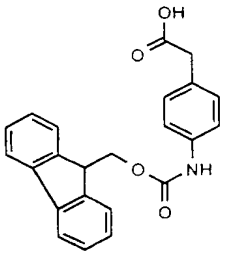
The completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test. The product was then washed and deprotected as described in Step 2. This functionalised resin was then ready for peptide synthesis.

Example 2

Step 1

Peptide Fmoc-Papa-OH Coupling

2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (15.4g, 47.5mmol, 9.50 equiv.) was added to a clean dry 250ml flask equipped with a magnetic stirrer. To this was added a protected amino acid Fmoc-Papa-OH of structure



(18.9g, 50.0mmol, 10.0 equiv.) and the reagents dissolved in DMF (70ml). After chilling the solution to 0-5°C, diisopropylethylamine (DIPEA) was added (7.75g, 10.4ml, 60.0mmol, 12 equiv.) whilst keeping the solution below 5°C. The mixture was stirred for 10 minutes at 0-5°C and then added to the resin from Example 1 under a slight

nitrogen/argon pressure, together with a DMF (5ml) rinse and stirring. The completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test. The product was then washed and deprotected as described in Example 1 Step 2 above. Fmoc-Papa-OH was obtained as follows:-

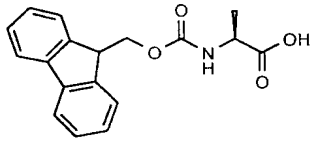
Fmoc-Papa-OH

To a slurry of 4-aminophenylacetic acid (Papa) (30.0g, 19.5mmole, 1.0 equiv.) in acetone (600ml) and aqueous sodium bicarbonate (36.13g, 428mmole, 2.2 equiv. in 600ml water) was added 9-fluorenylmethyl-succinimidyl carbonate (Fmoc-OSu) (70.3g, 204mmole, 1.05 equiv.) with stirring. After stirring overnight at room temperature the resulting slurry was acidified with concentrated hydrochloric acid (42.3ml, 486mmole, 2.5 equiv.) and the product extracted at 45°C into n-butyl acetate (2 x 600ml) when the organic extract was washed twice at 45°C with water (2 x 300ml), distilled under vacuum on a steam bath to 450ml and crystallised over 2 hours to room temperature. The crystalline product was isolated and washed twice with n-butyl acetate (2 x 120ml) and dried at 60°C in a vacuum oven. Yield 67.2g (92%)

Step 2

Fmoc-Ala-OH Coupling

In the next stage, HOBt (9.10g, 66.7mmol, 13.3 equiv.) and Fmoc protected alanine (Fmoc-Ala-OH) of formula



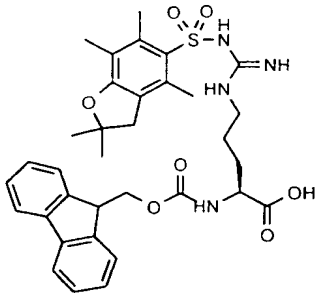
(16.6g, 50.0mmol, 10.0equiv.) was added to a clean dry 250ml flask equipped with a magnetic stirrer. The reagents were then dissolved in DMF (60ml) and chilled as before to 0-5°C. DIC (7.01g, 8.70ml, 55.0mmol, 11 equiv.) was added with stirring (10 minutes) whilst keeping the solution below 5°C.

The solution was then added to the product of Step 1 under a slight nitrogen/argon pressure, and rinsed and stirred in the presence of DMF as described in Step 1. The completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test. The product was washed with DMF (5 x 100ml). Acetic anhydride (10% v/v in DMF, 100ml) was added and the mixture stirred for 1 hour. This capped any residual aniline groups. There followed further washing with DMF, deprotection with piperidine in DMF and further DMF washing as described above.

Step 3

Fmoc-Arg(Pbf)-OH Coupling

In this coupling, HOBt (9.10g, 66.7mmol, 13.3 equiv.) was used together with Fmoc-Arg(Pbf)-OH (33.9g, 50.0mmol, 10.0 equiv.) of formula



dissolved in DMF (80ml) as the starting material. The solution was cooled to between 0-5°C and DIC (7.01g, 8.70ml, 55.0mmol, 11 equiv.) added whilst keeping the solution below 5°C with stirring for 5 minutes. The product was added to the resin of Step 2 under a slight pressure of nitrogen/argon with a DMF (5ml) rinse and stir.

After 5 minutes extra DMF (30ml) was charged as required to mobilise the resin and this mixture was left for 1 hour. The completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test. Washing with DMF and deprotection with piperidine in DMF was effected as described above in Example 1 Step 2.

Step 4

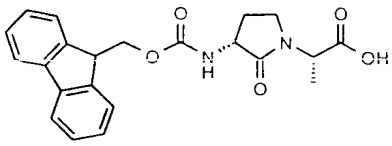
Fmoc-Ala-OH Coupling

Step 2 was repeated in order to link a further alanine residue to the developing peptide.

Step 5

Fmoc-Lactam Coupling

A further coupling reagent sample was prepared by dissolving HOBt (9.10g, 66.7mmol, 13.3 equiv.) and (2S)-2-[(3R)-3-(N-[9-fluorenylmethyloxycarbonyl]amino-2-oxopyrrolidin-1-yl]propionic acid (19.9g, 50.0mmol, 10.0 equiv.) of formula



obtained as described in WO 97/31023, in DMF (70ml) in a clean dry 250ml flask equipped with a magnetic stirrer. After chilling to 0-5°C, DIC (7.01g, 8.70ml, 55.0mmol, 11 equiv.) was added with stirring for 10 minutes whilst keeping the solution below 5°C.

The solution was then charged to the product of Step 4 under a small pressure of argon/nitrogen with a DMF (5ml) rinse and stir. After 5 minutes, extra DMF (30ml) was added to mobilise the resin which was then left for 1 hour. The completion of the reaction was checked by removing approximately 5mg of beads from the resin bed and carrying out a Kaiser test.

Solvent was then removed under reduced pressure and the product treated with DMF and piperidine in DMF as described in Example 1 Step 2. The resin was then filtered under vacuum.

Step 6

Fmoc-Ala-OH Coupling

A further Fmoc protected alanine residue was coupled next by repeating the process of Step 2 above.

Step 7

Fmoc-Arg(Pbf)-OH Coupling

Step 3 was repeated.

Step 8

Fmoc-Ala-OH Coupling

The coupling of Step 2 was repeated.

Step 9

Phv-OH Coupling

In this coupling, the initial solution comprised HOBt (9.10g, 66.7mmol, 13.3 equiv.) and 5-phenylvaleric acid (Phv-OH) (9.00g, 50.0mmol, 10.0 equiv.) dissolved in DMF (70ml).

After chilling the solution to 0-5°C, DIC (7.01g, 8.70ml, 55.0mmol, 11 equiv.) was added with stirring for 10 minutes whilst keeping the solution below -5°C. The solution was applied to the resin of Step 8 which had been kept under the slight nitrogen/argon pressure, together with a rinse solvent comprising DMF (5 ml) with stirring. After 5 minutes, extra DMF (80ml) was added to mobilise the resin which was

then left for 1 hour. A Kaiser test as described above was effected to check completion of the reaction and the solvent was removed under reduced pressure.

After slurry washing the product, first with DMF (10 x 175ml) and then with diethyl ether (5 x 175ml), it was dried using a nitrogen purge on a sintered funnel for 10 minutes. Further drying was carried out at 40°C in a vacuum oven overnight to constant weight.

Example 4

Cleavage and Analysis of product

The resin product from Example 3 (6.6g) was transferred to a 250ml flask and triethylsilane in trifluoroacetic acid (TFA) (10% v/v, 132ml) added rapidly in one portion. The mixture was stirred for 2 hours, then filtered and the residue washed with triethylsilane in TFA (10% v/v, 2 x 50ml). After evaporation of the filtrate to dryness on a rotary evaporator at 40°C, diethyl ether (100ml) was added and the mixture stirred vigorously for 14 hours to precipitate the peptide. The precipitate was filtered on a sintered funnel, washed with diethyl ether (3 x 30ml) and air dry on the sinter for 5 minutes. Further drying was carried out overnight at 40°C in a vacuum oven. The target peptide (Scheme 1) was obtained.

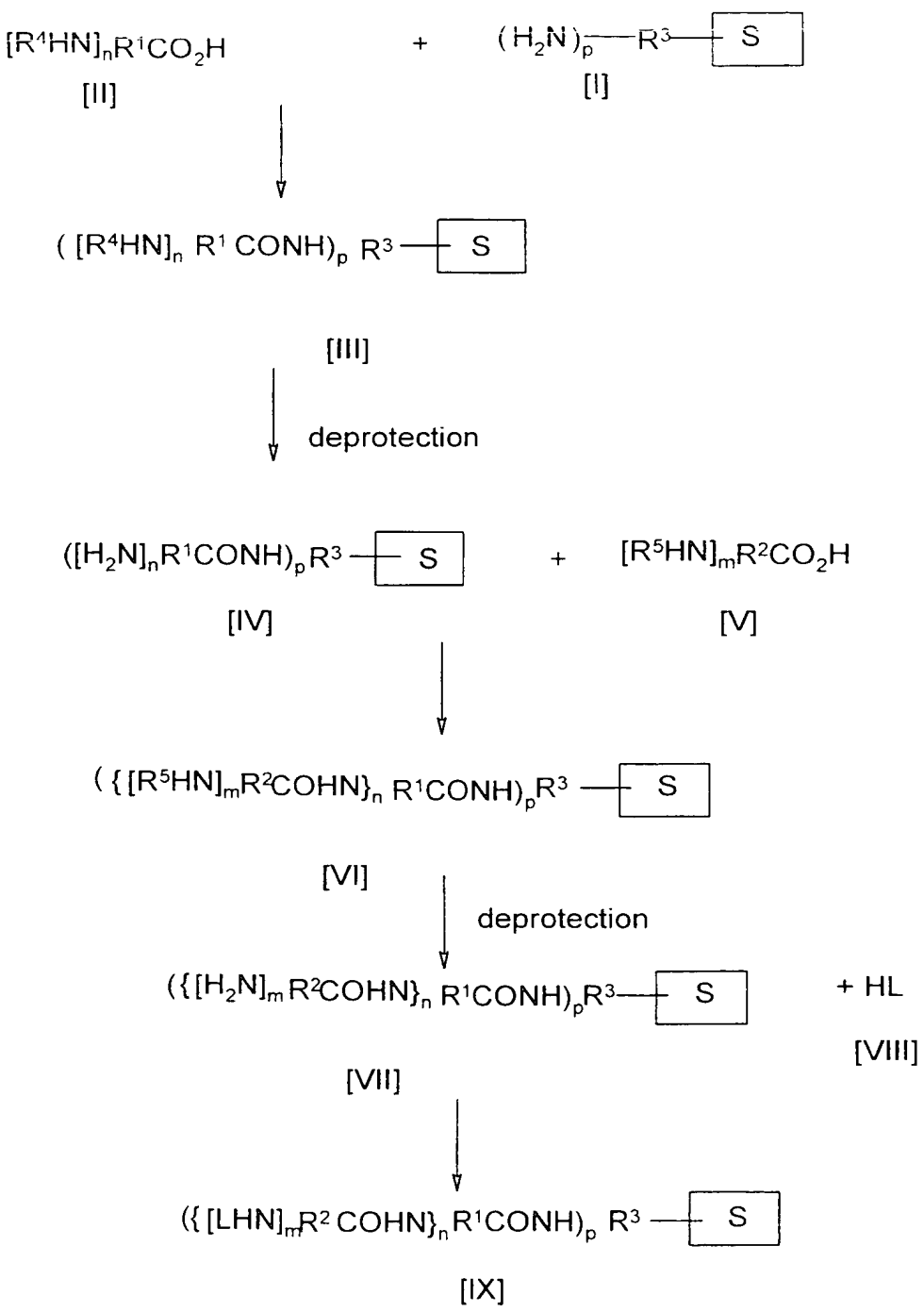
In an alternative cleavage process, the resin product from Example 3 (20.0g, 2.271mmol) was added to a reactor and a slight nitrogen pressure applied. Triethylsilane (40.0ml) and di-*n*-butyl ether (40.0ml) were added and the contents were agitated at 0°C. Cooling was maintained in order to control the exotherm of the reaction and to limit impurity formation. TFA (300ml) was added over a period of 1 hour and the mixture then agitated for 16 hours at 0°C.

The resultant suspension was then filtered under vacuum, washed twice with TFA (100ml), and the wash materials and the filtrate combined. TFA was then distilled off under reduced pressure at 25°C until only a trickle distilled.

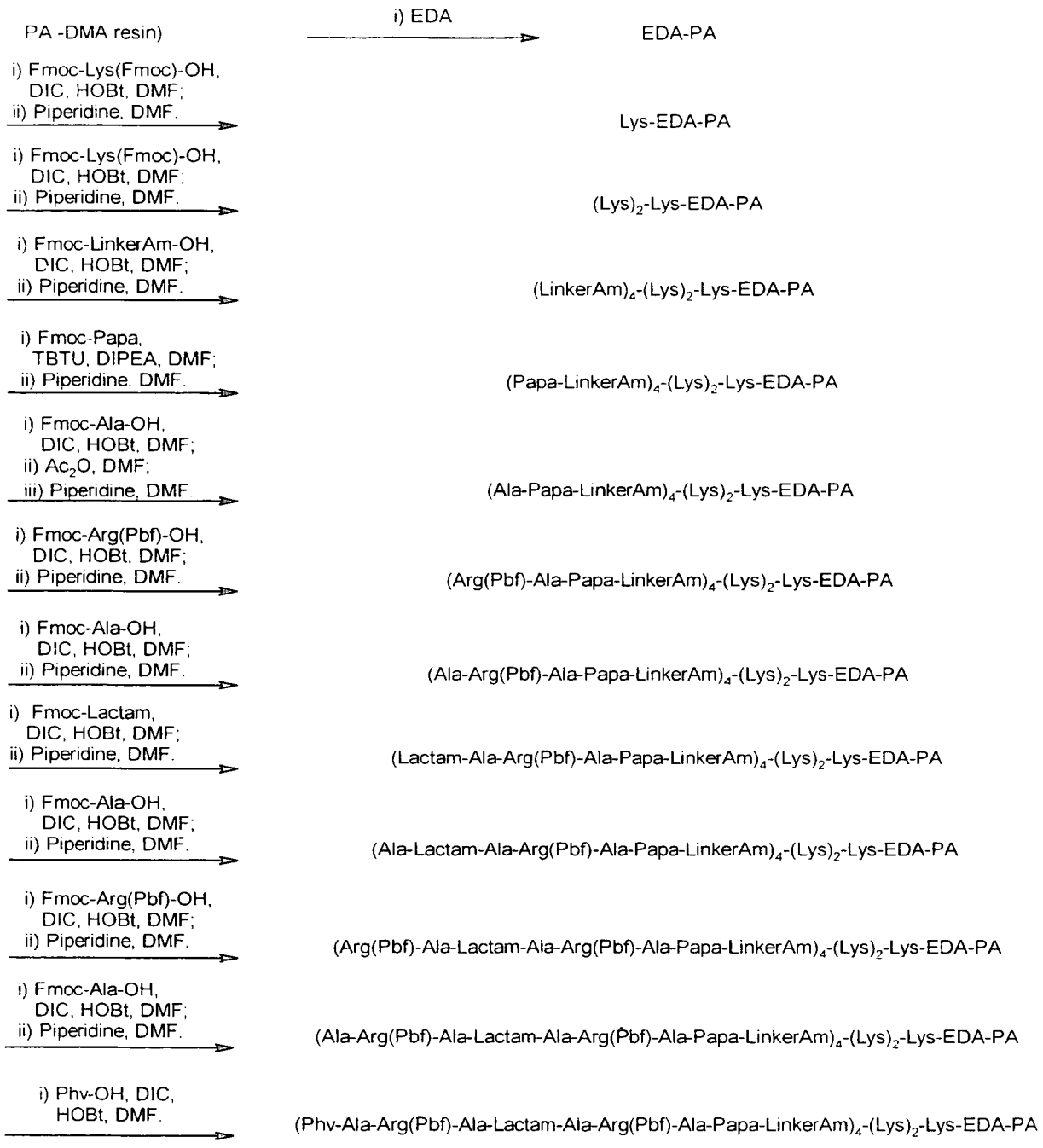
The concentrate obtained was added to rapidly agitated diiso-propyl ether (400ml) and the mixture stirred for 30 minutes at ambient temperature. The precipitate was collected by filtration, washed with diiso-propyl ether (100ml) and the cake deliquored. The wash and deliquor step was repeated once and the product dried at 40°C in a vacuum oven for 2 hours.

9.97g of peptide was obtained.

Scheme 1


[R4HN]nR1CO2H
[II]
+
(H2N)p—R3—S
[I]
([R4HN]n R1 CONH)p R3—S
[III]
deprotection
([H2N]nR1CONH)pR3—S
[IV]
+
[R5HN]mR2CO2H
[V]
({[R5HN]mR2COHN}n R1CONH)pR3—S
[VI]
deprotection
({[H2N]m R2COHN}n R1CONH)pR3—S
+ HL
[VIII]
[VII]
({[LHN]mR2 COHN}n R1CONH)p R3—S
[IX]

Scheme 2


PA -DMA resin)
i) EDA
EDA-PA
i) Fmoc-Lys(Fmoc)-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
Lys-EDA-PA
i) Fmoc-Lys(Fmoc)-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Lys)2-Lys-EDA-PA
i) Fmoc-LinkerAm-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Papa, TBTU, DIPEA, DMF; ii) Piperidine, DMF.
(Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Ala-OH, DIC, HOBt, DMF; ii) Ac2O, DMF; iii) Piperidine, DMF.
(Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Arg(Pbf)-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Ala-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Lactam, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Lactam-Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Ala-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Ala-Lactam-Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Arg(Pbf)-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Arg(Pbf)-Ala-Lactam-Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Fmoc-Ala-OH, DIC, HOBt, DMF; ii) Piperidine, DMF.
(Ala-Arg(Pbf)-Ala-Lactam-Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA
i) Phv-OH, DIC, HOBt, DMF.
(Phv-Ala-Arg(Pbf)-Ala-Lactam-Ala-Arg(Pbf)-Ala-Papa-LinkerAm)4-(Lys)2-Lys-EDA-PA

Scheme 2 (continued)

i) TFA / triethylsilane (90:10, 20 vol, 0 °C);
ii) Diethyl ether (precip.).

Phv-Ala-Arg-Ala-Lactam-Ala-Arg-Ala-Papa-NH_2 .2 TFA

iii) HPLC (MeCN, H_2O, NH_4OAc, HOAc, pH 5).

Phv-Ala-Arg-Ala-Lactam-Ala-Arg-Ala-Papa-NH_2 .2 AcOH

PA = Polyamide sarcosine resin, solid support
EDA = Ethylenediamine
Lys = Lysine (used twice to increase resin capacity x 4)
LinkerAm = Acid labile linkage agent
Phv-OH = 5-Phenylvaleric acid

CLAIMS

1. A method for preparing a solid support material for carrying out a chemical reaction, said method comprising the following steps:

(i) reacting an amino functionalised solid material with a carboxylic acid having at least two similarly protected amino groups to form amide bonds between them,

(ii) removing protecting groups in a single step,

(iii) optionally repeating steps (i) and (ii) one or more times using the product of the preceding step as the amino functionalised solid material, and

(iv) connecting a linkage agent to at least some of the free NH_2 groups of the product.

2. A method according to claim 1 wherein the said carboxylic acid comprises an amino acid.

3. A method according to claim 2 wherein the amino acid is lysine or ornithine.

4. A method according to claim 1 or claim 2 wherein the amino functionalised solid material is obtained by reacting an acid or ester substituted support with a compound of formula (X)

$$H_2N\text{-}[(R^8)_zNH)]_y(R^{8'})_x\text{-}NH_2$$

(X)

where x is an integer of 2 or more, y is 0 or an integer of 1 or more, and each group z is independently 2 or more, and each R^8 and $R^{8'}$ are the same or different and are optionally substituted divalent hydrocarbyl groups.

5. A method according to claim 4 wherein the compound of formula (X) is ethylene diamine.

6. A solid support material obtainable by the method of any one of the preceding claims.

7. A solid support material comprising a compound of formula (IX)

$$(\{[LHN]_mR^2COHN\}_nR^1CONH)_p\ R^3 - \boxed{S}$$

[IX]

wherein S is a solid polymer core;

R^1 is a organic moiety with n + 1 available points for bonding;
R^2 is an organic moiety with m + 1 available points for bonding;
R^3 is either a bond or an organic bridging group;
R^4 and R^5 are protecting groups;
L is a linkage agent, or a protected form thereof;
p is an integer of 1 or more, provided that p is 1 when R^3 is a bond; and
n and m are independently selected from integers of 2 or more.

8. A compound of formula (XIII)

XIII

wherein S, R^1, R^2, R^3, n, m and p are as defined in claim 7, and R^{20} is hydrogen or an amino protecting group.

9. A method for preparing a compound, which method comprises binding a reagent to a linkage agent of a support material according to claim 6 or claim 7, effecting one or more reaction steps to generate product, and thereafter cleaving said product from the support material.

10. A method according to claim 9 wherein the product is a therapeutic peptide.

11. A method according to claim 10, wherein the therapeutic peptide is

Phv-Ala-Arg-Ala-Lactam-Ala-Arg-Ala-Papa-NH_2

or a pharmaceutically acceptable salt thereof.

12. A method for preparing a peptide which comprises coupling a protected amino acid to a linkage agent immobilised on a solid support, deprotecting the amino acid and thereafter coupling a further protected amino acid to said first amino acid and repeating said process until the desired peptide is produced, and thereafter cleaving the peptide from the solid support, characterised in that, where the amino acid is a protected 4-aminophenyl acetic acid (PAPA), the coupling agent is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and the coupling is effected in the presence of diisopropylethylamine (DIPEA).

13. A method according to claim 12, wherein for couplings where the amino acid is other than a protected 4-aminophenyl acetic acid (PAPA) a coupling reagent comprising a carbodiimide is employed in the presence of a compound that forms an active ester.

14. A method according to claim 12 or 13, wherein the solid support comprises a solid support material according to claim 6 or claim 7.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02007

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K1/04 C07K14/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 884 327 A (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 16 December 1998 (1998-12-16) example 1	8
X	WO 95 00540 A (R WEBBER) 5 January 1995 (1995-01-05) figure 1	8
A	WO 95 00165 A (SELECTIDE CORPORATION) 5 January 1995 (1995-01-05) the whole document	1-14
X	WO 94 02506 A (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 3 February 1994 (1994-02-03) figure 3	8

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	13/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 360 062 A (BAYER AG) 28 March 1990 (1990-03-28) the whole document -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/02007

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 884327 A	16-12-1998	JP 11124396 A	11-05-1999
WO 9500540 A	05-01-1995	NONE	
WO 9500165 A	05-01-1995	AU 689116 B	26-03-1998
		AU 7114594 A	17-01-1995
		CA 2165780 A	05-01-1995
		EP 0751779 A	08-01-1997
		JP 9500111 T	07-01-1997
		NZ 268292 A	22-09-1997
		US 5635598 A	03-06-1997
WO 9402506 A	03-02-1994	AU 4715493 A	14-02-1994
		DE 69315842 D	29-01-1998
		DE 69315842 T	09-04-1998
		EP 0652896 A	17-05-1995
		ES 2111167 T	01-03-1998
		US 5882645 A	16-03-1999
EP 360062 A	28-03-1990	DE 3831708 A	22-03-1990
		JP 2115226 A	27-04-1990
		US 5049651 A	17-09-1991

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SMC 70539/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02007	25/05/2000	04/06/1999
International Patent Classification (IPC) or national classification and IPC C07K1/04		
Applicant AVECIA LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/12/2000	04.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer G. Willière Telephone No. +49 89 2399 8548

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02007

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22	as originally filed	

Claims, No.:

1-14	with telefax of	23/05/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V
Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
Re Item V

1. Reference is made to the following documents:

   D1: EP-A-0 884 327 (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 16 December 1998 (1998-12-16)
   D2: WO 95 00540 A (R WEBBER) 5 January 1995 (1995-01-05)
   D3: WO 94 02506 A (THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON) 3 February 1994 (1994-02-03)
   D4: EP-A-0 360 062 (BAYER AG) 28 March 1990 (1990-03-28)

2. The present application relates to a method for preparing a solid support material for carrying out a chemical reaction characterized by reacting an amino functionalised solid support with an amino acid having at least two amino moieties being similarly protected, deprotecting and attaching a linkage agent to said deprotected amino functions.

3. D4 discloses a process for preparing co(polyamides) by condensing e.g. diamine and dicarboxylic acids. D4 is thus not believed to have a particular relevance for the presently claimed method (see paragraph 2).

4. The disclosure of D1 (referring to polymers for use as carriers in targeted drug delivery) differs from what is presently claimed by reacting an amino functionalised solid support (see (R)-NH-Gly-NHBoc) with an amino acid being differently protected (in order to build up different dendrons). The linkage agent is represented by free amino groups.

5. D2 discloses a carrier for delivery of biologically active components to an organism, which is principally built up in a similar manner than does the support of the present application, i.e. a series of peptides having a pair of ends capable of serving as branch sites (e.g. Lys) bound to an resin in order to prepare a branched carrier bearing a high number of terminal linkage agents.

6. D3 (also referring to polymers for use as carriers in targeted drug delivery) again refers to the same principle then do D1, D2 and the present application: a branched terminal functional groups bearing solid supports for carrying out a chemical reaction (here the linkage of a peptide antigen thereto). However D3 introduces a novel feature, which is the lipid anchor having a spacer function.

7. The problem underlying the present alleged invention is thus the provision of a multi-functionalised solid support for carrying out a chemical reaction having an increased loading capacity.

   Documents D1 to D3 thus refer to a different field. Moreover no motivation can be identified from the teaching of said documents which would obviously lead the skilled person to a solid support according to the present application which properties are modified so as to increase its loading capacity.

Re Item VII

Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D3 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

No unified criteria exists in the PCT as to the acceptance of documents being "incorporated by reference" in an application. The EPO for example request these references to be deleted from the description in case such publications (and other material) are not essential for carrying out the invention. If, however, it turns out that said publications and other materials are in fact essential for carrying the invention, the EPO requests them/it to be expressly incorporated into the description. Such incorporation is, however, subject to the restrictions as listed in the decision T669/90, OJ 10/93, 616, of the EPO's Technical Board of Appeal.

SMC 70539

CLAIMS

1. A method for preparing a solid support material for carrying out a chemical reaction, said method comprising the following steps:

(i) reacting an amino functionalised solid material with a carboxylic acid having at least two similarly protected amino groups to form amide bonds between them,

(ii) removing protecting groups in a single step,

(iii) optionally repeating steps (i) and (ii) one or more times using the product of the preceding step as the amino functionalised solid material, and

(iv) connecting a linkage agent to at least some of the free NH_2 groups of the product.

2. A method according to claim 1 wherein the said carboxylic acid comprises an amino acid.

3. A method according to claim 2 wherein the amino acid is lysine or ornithine.

4. A method according to claim 1 or claim 2 wherein the amino functionalised solid material is obtained by reacting an acid or ester substituted support with a compound of formula (X)

$$H_2N\text{-}[(R^{8})_z NH)]_y(R^{8'})_x\text{-}NH_2$$

(X)

where x is an integer of 2 or more, y is 0 or an integer of 1 or more, and each group z is independently 2 or more, and each R^{8} and $R^{8'}$ are the same or different and are optionally substituted divalent hydrocarbyl groups.

5. A method according to claim 4 wherein the compound of formula (X) is ethylene diamine.

6. A solid support material obtainable by the method of any one of the preceding claims.

7. A solid support material comprising a compound of formula (IX)

$$(\{[LHN]_m R^2 COHN\}_n R^1 CONH)_p\ R^3 - \boxed{S}$$

[IX]

 AMENDED SHEET

wherein S is a solid polymer core;

R^1 is a organic moiety with n + 1 available points for bonding:

R^2 is an organic moiety with m + 1 available points for bonding;

R^3 is either a bond or an organic bridging group;

L is a linkage agent, or a protected form thereof;

p is an integer of 1 or more, provided that p is 1 when R^3 is a bond; and

n and m are independently selected from integers of 2 or more.

8. A compound of formula (XIII)

$$(\{[R^{20}HN]_mR^2CONH\}_nR^1CONH)_pR^3 — \boxed{S}$$

XIII

wherein S, R^1, R^2, n, m and p are as defined in claim 7, R^3 is either a bond or an organic bridging group formed by reaction of acid or ester functionalities at the surface of S with an amine of formula (X)

$$H_2N\text{-}[(R^8)_zNH)]_y(R^{8'})_x\text{-}NH_2$$

(X)

where x is an integer of 2 or more, y is 0 or an integer of 1 or more, each group z is independently 2 or more, and each R^8 and $R^{8'}$ are the same or different and are optionally substituted divalent hydrocarbyl groups and R^{20} is hydrogen or an amino protecting group.

9. A method for preparing a compound, which method comprises binding a reagent to a linkage agent of a support material according to claim 6 or claim 7, effecting one or more reaction steps to generate product, and thereafter cleaving said product from the support material.

10. A method according to claim 9 wherein the product is a therapeutic peptide.

11. A method according to claim 10, wherein the therapeutic peptide is

Phv-Ala-Arg-Ala-Lactam-Ala-Arg-Ala-Papa-NH_2

or a pharmaceutically acceptable salt thereof.

12. A method for preparing a peptide which comprises coupling a protected amino acid to a linkage agent immobilised on a solid support, deprotecting the amino acid and thereafter

SMC 70539

coupling a further protected amino acid to said first amino acid and repeating said process until the desired peptide is produced, and thereafter cleaving the peptide from the solid support, characterised in that, where the amino acid is a protected 4-aminophenyl acetic acid (PAPA), the coupling agent is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and the coupling is effected in the presence of diisopropylethylamine (DIPEA).

13. A method according to claim 12, wherein for couplings where the amino acid is other than a protected 4-aminophenyl acetic acid (PAPA) a coupling reagent comprising a carbodiimide is employed in the presence of a compound that forms an active ester.

14. A method according to claim 12 or 13, wherein the solid support comprises a solid support material according to claim 6 or claim 7.

CLAIMS

1. A method for preparing a solid support material for carrying out a chemical reaction, said method comprising the following steps:

(i) reacting an amino functionalised solid material with a carboxylic acid having at least two similarly protected amino groups to form amide bonds between them,

(ii) removing protecting groups in a single step,

(iii) optionally repeating steps (i) and (ii) one or more times using the product of the preceding step as the amino functionalised solid material, and

(iv) connecting a linkage agent to at least some of the free NH_2 groups of the product.

2. A method according to claim 1 wherein the said carboxylic acid comprises an amino acid.

3. A method according to claim 2 wherein the amino acid is lysine or ornithine.

4. A method according to claim 1 or claim 2 wherein the amino functionalised solid material is obtained by reacting an acid or ester substituted support with a compound of formula (X)

$$H_2N\text{-}[(R^8)_zNH)]_y(R^{8'})_x\text{-}NH_2$$

(X)

where x is an integer of 2 or more, y is 0 or an integer of 1 or more, and each group z is independently 2 or more, and each R^8 and $R^{8'}$ are the same or different and are optionally substituted divalent hydrocarbyl groups.

5. A method according to claim 4 wherein the compound of formula (X) is ethylene diamine.

6. A solid support material obtainable by the method of any one of the preceding claims.

7. A solid support material comprising a compound of formula (IX)

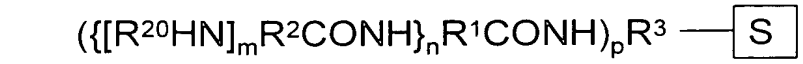
$$(\{[LHN]_mR^2COHN\}_nR^1CONH)_p\ R^3 - \boxed{S}$$

[IX]

wherein S is a solid polymer core;

R^1 is a organic moiety with n + 1 available points for bonding:
R^2 is an organic moiety with m + 1 available points for bonding;
R^3 is either a bond or an organic bridging group;
R^4 and R^5 are protecting groups;
L is a linkage agent, or a protected form thereof;
p is an integer of 1 or more, provided that p is 1 when R^3 is a bond; and
n and m are independently selected from integers of 2 or more.

8. A compound of formula (XIII)



XIII

wherein S, R^1, R^2, R^3, n, m and p are as defined in claim 7, and R^{20} is hydrogen or an amino protecting group.

9. A method for preparing a compound, which method comprises binding a reagent to a linkage agent of a support material according to claim 6 or claim 7, effecting one or more reaction steps to generate product, and thereafter cleaving said product from the support material.

10. A method according to claim 9 wherein the product is a therapeutic peptide.

11. A method according to claim 10, wherein the therapeutic peptide is

Phv-Ala-Arg-Ala-Lactam-Ala-Arg-Ala-Papa-NH_2

or a pharmaceutically acceptable salt thereof.

12. A method for preparing a peptide which comprises coupling a protected amino acid to a linkage agent immobilised on a solid support, deprotecting the amino acid and thereafter coupling a further protected amino acid to said first amino acid and repeating said process until the desired peptide is produced, and thereafter cleaving the peptide from the solid support, characterised in that, where the amino acid is a protected 4-aminophenyl acetic acid (PAPA), the coupling agent is 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and the coupling is effected in the presence of diisopropylethylamine (DIPEA).

13. A method according to claim 12, wherein for couplings where the amino acid is other than a protected 4-aminophenyl acetic acid (PAPA) a coupling reagent comprising a carbodiimide is employed in the presence of a compound that forms an active ester.

14. A method according to claim 12 or 13, wherein the solid support comprises a solid support material according to claim 6 or claim 7.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For Receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* SMC 70539/WO

Box No. I TITLE OF INVENTION

Process for the preparation of supports for solid phase synthesis

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Avecia Limited
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☐ This person is also inventor.

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State *(that is, country)* of nationality:
GB

State *(that is, country)* of residence:
GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BROWN, Richard John
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United Kingdom

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:
GB

State *(that is, country)* of residence:
GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

REVELL, Christopher
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Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III **FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MONTGOMERY, Francis Joseph
Silk Road Business Park
Macclesfield Works
Macclesfield
Cheshire SK10 2NA
United Kingdom

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: JR IE

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HARRIS, Craig Steven
Mereside
Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WELLINGS, Donald Alfred
Gadbrook Park
Rudheath
Northwich
Cheshire SW9 7RA
United Kingdom

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ **AE** United Arab Emirates
☒ **AL** Albania
☒ **AM** Armenia
☒ **AT** Austria
☒ **AU** Australia
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina
☒ **BB** Barbados
☒ **BG** Bulgaria
☒ **BR** Brazil
☒ **BY** Belarus
☒ **CA** Canada
☒ **CH and LI** Switzerland and Liechtenstein
☒ **CN** China
☒ **CR** Costa Rica
☒ **CU** Cuba
☒ **CZ** Czech Republic
☒ **DE** Germany
☒ **DK** Denmark
☒ **DM** Dominica
☒ **EE** Estonia
☒ **ES** Spain
☒ **FI** Finland
☒ **GB** United Kingdom
☒ **GD** Grenada
☒ **GE** Georgia
☒ **GH** Ghana
☒ **GM** Gambia
☒ **HR** Croatia
☒ **HU** Hungary
☒ **ID** Indonesia
☒ **IL** Israel
☒ **IN** India
☒ **IS** Iceland
☒ **JP** Japan
☒ **KE** Kenya
☒ **KG** Kyrgyzstan
☒ **KP** Democratic People's Republic of Korea
☒ **KR** Republic of Korea
☒ **KZ** Kazakhstan
☒ **LC** Saint Lucia
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho
☒ **LT** Lithuania
☒ **LU** Luxembourg
☒ **LV** Latvia
☒ **MA** Morocco
☒ **MD** Republic of Moldova
☒ **MG** Madagascar
☒ **MK** The former Yugoslav Republic of Macedonia
☒ **MN** Mongolia
☒ **MW** Malawi
☒ **MX** Mexico
☒ **NO** Norway
☒ **NZ** New Zealand
☒ **PL** Poland
☒ **PT** Portugal
☒ **RO** Romania
☒ **RU** Russian Federation
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia
☒ **SK** Slovakia
☒ **SL** Sierra Leone
☒ **TJ** Tajikistan
☒ **TM** Turkmenistan
☒ **TR** Turkey
☒ **TT** Trinidad and Tobago
☒ **TZ** United Republic of Tanzania
☒ **UA** Ukraine
☒ **UG** Uganda
☒ **US** United States of America
☒ **UZ** Uzbekistan
☒ **VN** Viet Nam
☒ **YU** Yugoslavia
☒ **ZA** South Africa
☒ **ZW** Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ DZ Algeria
X MZ Mozambique
☒ AG Antigua and Barbuda

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "**patent of addition**," or "**certificate of addition**," or if, in Box No. V, the name of the United States of America is accompanied by an indication "**continuation**" or "**continuation-in-part**": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

Continuation of Box IV

FAWKES, David Melville
MAYALL, John
NELSON, Michael Andrew
PUGSLEY, Roger Graham
SCHMITT, Maja
SHELLER, Alan

All of Intellectual Property Group, Avecia Limited, PO Box 42, Hexagon House, Blackley, Manchester M9 8ZS, United Kingdom

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 04/06/99 4 June 1999	9912911.6	GB		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)* :

ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 05
description (excluding sequence listing part)	: 22
claims	: 03
abstract	: 01
drawings	: 00
sequence listing part of description	: 00
Total number of sheets	: 31

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

For Avecia Limited, R.J. Brown, F.J. Montgomery, C.S. Harris & D.A. Wellings

REVELL, Christopher

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: SMC 70539/WO

Applicant: Avecia Limited

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . GBP 55 | T

2. SEARCH FEE . . . . . GBP 638 | S

International search to be carried out by EPO

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 31 sheets.

first 30 sheets . . . . . GBP 264 | b1

1 (remaining sheets) x 6 (additional amount) = GBP 6 | b2

Add amounts entered at b1 and b2 and enter total at B . . . . GBP 270 | B

Designation Fees

The international application contains ALL designations.

8 (number of designation fees payable *(maximum 8)*) x 56 (amount of designation fee) = GBP 448 | D

Add amounts entered at B and D and enter total at I . . . . . GBP 718 | I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . GBP 22 | P

5. TOTAL FEES PAYABLE . . . . . GBP 1433 | TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

- ☒ authorization to charge deposit account (see below)
- ☐ bank draft
- ☐ coupons
- ☐ cheque
- ☐ cash
- ☐ other *(specify)*:
- ☐ postal money order
- ☐ revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ GB

☒ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☒ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

D02944	24 May 2000	G Terry
Deposit Account No.	Date *(day month/year)*	Signature